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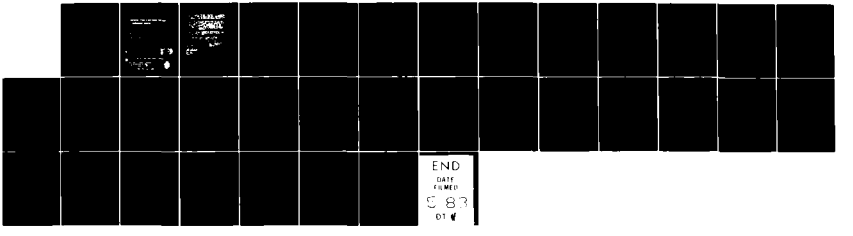

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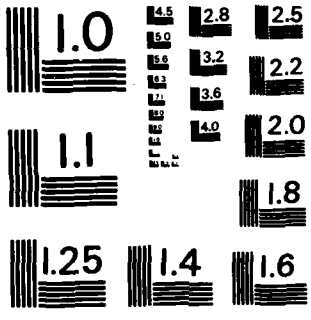

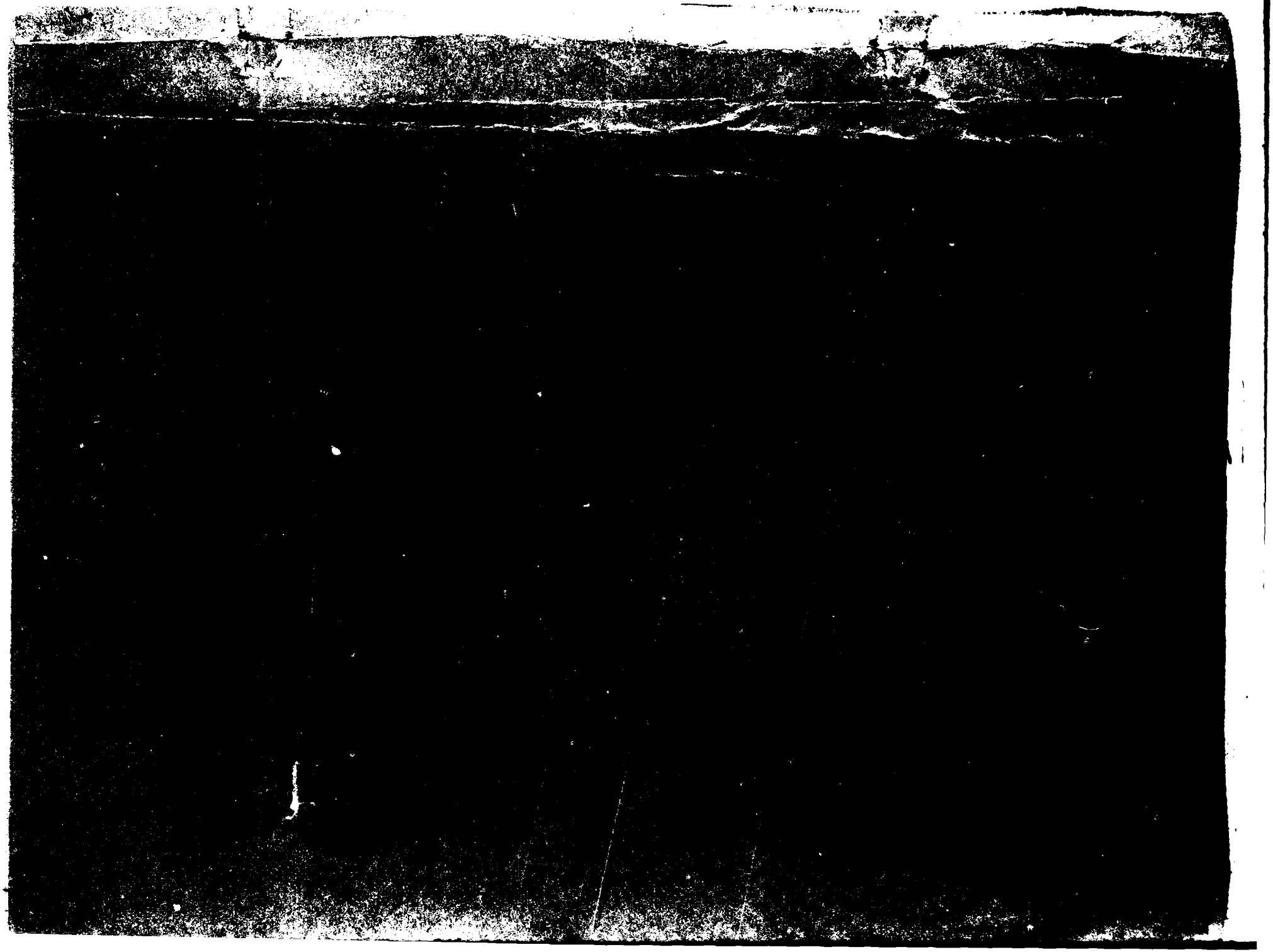

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A series of temperature cycling experiments during radiofrequency radiation (RFR) exposure of rats was performed. This type of exposure procedure permits electromagnetic energy to be introduced into biological systems while the systems are maintained at physiologically acceptable temperatures. Experiments were carried out at various average power densities (50-200 mW/cm^2), using continuous wave (CW) and pulsed radiation while the carrier frequency was maintained at 2.06 GHz. Single-day RFR exposures produced no observable effect on temperature regulation of rats in terms of heat-dissipation efficiency. Repeated exposure

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20. ABSTRACT (Continued)

of rats during several months revealed that the heat-dissipation time (D) gradually increased, indicating reduced efficiency. After a prolonged resting period, however, the rat's ability to dissipate heat showed considerable improvement. No significant difference in thermoregulation was observed when CW and pulsed RFR exposures at pulse durations of 1-10 ms were compared.

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TEMPERATURE CYCLING IN RATS EXPOSED REPETITIVELY TO RADIOFREQUENCY RADIATION

INTRODUCTION

Current use of radiofrequency radiation (RFR) is so widespread in our society that most of the population is exposed in various modes and patterns. Common sources include AM and FM broadcasting, radios, CB radios, microwaves in household use, and clinical therapy. The current technical trend in industrial and military development indicates even more extensive RFR applications in the future. Therefore, it is imperative that we be well aware of the biological effects exerted by RFR on humans and other species. An extensive research effort has been carried out on an international scale, but the results are ambiguous and often misleading. Several reviews and articles on this subject are available in the literature (1-5), so we will not dwell on it here. However, we will analyze the reasons for and propose solutions to some of the problems inherent in research approaches and field applications. We will also briefly consider the biological effects of RFR exposure.

A rapidly accumulating literature presents claims of both thermal (1,3) and nonthermal (3,5) effects of RFR on biological systems. Certain effects such as RFR-induced slow responses of Aplysia pacemaker neurons are thought to be nonthermal in nature (6). The thermal vs. nonthermal controversy surrounding the RFR-induced effects on the cochlear microphonics has been well summarized (3). McAfee (7) has even voiced the presently technically untestable hypothesis that all RFR-induced effects are due to very localized hot spots; thus, all are thermal. Nonthermal processes, however, are equally conceivable (see THEORETICAL CONSIDERATIONS and Adey (1) and Meyers and Ross (3)).

The terms "thermal" and "nonthermal" are loosely characterized in the literature. Basically, a process is considered to be thermal when the energy absorbed by an entity is transformed into heat at the absorption site. Heat can be expressed as an increase of random-movement velocities of molecules at the site. However, electromagnetic field energy could produce changes in the natural order or randomness of molecular motions without an increase of mean velocity; such changes could result in micro- and macrostructural disorganization (8). This could be expressed also in terms of structural entropy change at the macrostructure level, which in turn can be related to the stability of biological systems. Also, RFR can potentially modify the ordered molecular and ionic distributions in solutions in terms of concentration patterns and gradients.

The constituents and configurations of the various molecular species comprising the biological entity determine the specific frequencies at which RFR absorption can occur. Conceptually, such absorption can be characterized as nonthermal. If the energy is transformed locally into heat, however, distinguishing between nonthermal and thermal effects is difficult. Characterizing an RFR interaction with a biological entity as nonthermal presupposes that the interaction would produce a discrete or frequency-specific reaction that is experimentally distinguishable from general heating effects. However, these

two processes may occur simultaneously. Data in the literature indicate a dominance of thermal effects of in vivo and in vitro RFR exposure of biological systems (1-5). Exposure of a biological entity may produce multicomponent effects that are reflected in alterations of physiological processes, such as the regulation of blood pressure, heart rate, and respiratory rate.

In any study of RFR effects on animals, the experimental procedures should afford control, or at least definition, of thermal effects. Therefore, a large amount of energy (E) must be introduced into the biological system without exceeding the tolerable temperature range of the exposed species. Since this issue has been a stumbling block in most RFR animal experiments in the past, a new approach is needed.

The control of body temperature depends upon the coordinated activities of many bodily organs. While maintenance of a stable, deep-body temperature hinges on the function of the hypothalamic region of the brain, other areas of the central nervous system and several autonomic physiological functions also participate. Mammalian thermoregulation requires not only central temperature sensors and neuron assemblies, but the integrity of the system as a whole. The thermoregulatory process is also affected by the route transferring thermal energy to the body. The heat transfer would be different for a hot-air environment, involving many surface sensors, than for RFR which depends on the depth of penetration and distribution in the body. Alterations of thermoregulatory rate and efficiency could therefore reflect changes in the overall process of thermoregulation (9,10).

THEORETICAL CONSIDERATIONS

In analyzing a system that is multifunctional and has simultaneously proceeding processes, we have to consider how to resolve and discriminate the entities. One possible "handle" of such a systemic analysis is the phenomenon that the temperature or energy transfer of the system is an inherently reversible process. On the other hand, disorganization and displacement of structural patterns (e.g., ionic, atomic, molecular) is in practical terms irreversible. On theoretical grounds, therefore, we have a basis to achieve experimental conditions where a nonreversible or slowly reversible process can obtain dominance over a more readily reversible one, since the irreversible or less reversible processes can be integrated. This scheme could be useful in observing and comparing the effects of pulsed and CW RFR.

Either a CW or pulsed wave form could conceivably induce transient changes at the molecular level. The timecourse of relaxation of the transient would determine the duration of the perturbed state. Repetition of RFR exposure with a frequency that exceeds the relaxation timecourse could augment the effect to the point where it could be directly measured or would be reflected in a physiological change that can be monitored. Also, pulsed and CW RFR could have differing abilities to induce such effects. An integration of the transient over time could permit us to

detect and possibly quantify differences (if present) in the effects of these two forms of RFR. Many (5) or all (7) RFR-induced changes may be due to localized heating effects, and certain irreversible thermal effects of microwave radiation (such as cataract formation) are well documented (11).

For completeness in this theoretical consideration of possible bio-effects, the possibility of irreversible mechanical and structural changes should be taken into regard. Bonds could be positioned and/or stressed so as to lower the activation energy for their participation in a chemical reaction. Also permanent structural changes at the subcellular level, such as the disruption of microtubules resulting from accumulations of unbound intracellular calcium, are not unthinkable, considering the putative effect of RFR on Ca^{++} binding (1, 3).

A simple formulation of the basic entities will illustrate this integration procedure in more abstract terms. Consider a system in which the energy content is E_1, at temperature T_1. After introduction of RFR fields, the energy content of the system is E_2 at temperature T_2. In terms of energy balance:

$$T_1(E_1) \underset{K_2}{\overset{\text{RFR}\ K_1}{\rightleftharpoons}} T_2(E_2) \underset{K_4}{\overset{K_3}{\rightleftharpoons}} [T_3(E_3)] \xrightarrow{K_5} X,$$

where X is an undefined disturbed state. Such a system reveals interesting characteristics. When the values of rate constants K_1 and K_3 are larger than those of K_2 and K_4, respectively, and the K_5 step is irreversible, the phenomenon is that the rate processes, when integrated, will produce an end product. Although the magnitude of the end product can be initially small, this product, when integrated with respect to time during many repetitive cycles of the process, may become the dominant feature of the system. A more detailed scheme would be desirable for quantitative analysis, but the present form was adequate to permit the development of a new experimental procedure.

In our experiment, RFR was applied intermittently to produce temperature cycles. Colonic temperature was continuously monitored with a Vitek thermistor probe during RFR exposure of rats. When the field is turned on, the energy input increases the rat's temperature to the desired level during timecourse R. When the field is turned off, the temperature returns to the basal level during a second timecourse, D. R plus D is defined as one cycle. If the cycling procedure is repeated several times at temperatures near the basal range, variations in R and D may be observed when the cycles are compared. However, if the rat's temperature is brought to an above-basal range (i.e., 38.5°-42°C) and the cycling procedure is repeated, the timecourses of the repeated cycles are consistent in individual rats.

Such a temperature-cycling procedure (a) permits exposing animals to a high degree of irradiation while maintaining the core temperature within acceptable ranges; (b) can demonstrate whether homeostatic-temperature regulating mechanisms are affected by multiple RFR exposures (This should be evident if temperature recovery time increases after repeated exposures.); and (c) allows physiological experiments to be carried out under defined thermal conditions, thus permitting comparison of different field effects.

EXPERIMENTAL METHODS

The CW fields were produced by a type 1326 generator (Cober Electronics, Inc.) and transmitted by a model H5001 antenna (American Electronic Laboratories, Inc.). Exposures were made under far-field conditions, and the incident power density of the field was determined with an electromagnetic radiation monitor (model 8316B, Narda Microwave Corp.) and a model 8323 probe. During exposures, the generator power was monitored constantly with a model 436A power meter (Hewlett-Packard). RFR exposures were made in an anechoic chamber (Rantec, division of Emerson Electric Company) at Brooks Air Force Base, San Antonio, Texas. The temperature and humidity of the chamber were monitored during all phases of the experiments.

For pulsed-exposure studies, fields were produced by a model 2852 S-band magnetron source (Cober Electronics, Inc.) and transmitted by a model 644 antenna (Narda Microwave Corp.). The power density of the field was determined and the generator power constantly monitored as for CW exposures. The pulsed exposures were carried out in an Eccosorb RF-shielded anechoic chamber (Emerson & Cuming, Inc.). The chamber temperature and humidity were constantly monitored.

The experiments were performed as follows: (1) An unanesthetized rat was placed in a holder and exposed to fields in an anechoic chamber while colonic temperature was monitored continuously with a Vitek 101 probe with an accuracy of $\pm 0.01^{\circ}C$; (2) when colonic temperature increased $1^{\circ}C$ above the initial value, exposure was discontinued; (3) when the temperature returned to the initial value, exposure was started again and continued until the temperature rise was $1^{\circ}C$ above the initial value; and (4) this procedure was repeated as long as desired. Several modifications in this procedure have been made. First, the cycling temperature may be greater than $1^{\circ}C$, so long as the peak temperature remains in a physiologically tolerable range. For example, we have cycled rats at 2°, 3°, and $4^{\circ}C$ above the basal temperature.

Temperature regulation at the basal range was not as responsive to exposure as it was at the 38° to $40^{\circ}C$ range, so most experiments were performed at a 38.5° to $39.5^{\circ}C$ cycling range. Alert rats were restless and mildly agitated during the first few cycles of field exposure but subsequently settled down, and the responsive temperature changes became regular and well defined. However, rats under ketamine anesthesia immediately started to cycle regularly, without an initial struggle.

Several drugs used in an effort to settle the rats changed the basal rat temperature and also modified its regulation. These drugs, therefore,

were discarded for routine experiments. Experiments on drug effects will be presented in separate reports.

Sprague-Dawley (female) rats weighing 200-300 g, were used in all experiments. The rats were held in quarantine for 10 days; some were then sacrificed for quality-control determinations. Subjects were housed in suspended stainless-steel wire cages, which were cleaned and sanitized weekly. Cage pans were cleaned and sanitized twice a week. The animals were fed a balanced commercial ration; water was available ad libitum. No solid food was fed for 18 hours preceding the exposure. To minimize diurnal effects on temperature cycling, radiation exposures were made at a fixed time, usually starting at 8 AM. Rats were placed in the holder and a preexposure waiting period was observed until the animal's temperature stabilized.

Prior to RFR exposure, the temperature-cycling phenomenon was studied during direct environmental-temperature exposure. The physiological processes affected under these conditions are not entirely the same as the processes affected by RFR; nevertheless, determination of the cooling time in the cycle was desirable. At least eight temperature cycles per day were performed to obtain meaningful experimental data. A thermal chamber (Blue M Electric Co.) that afforded regulation of both temperature and humidity was used to obtain data on effects of environmental-temperature changes. Temperature cycles were studied by placing the rat in the chamber until a specific colonic temperature was achieved (e.g., 38°C) and then removing it from the chamber until the desired basal temperature of the cycle (e.g., 37°C) occurred. This procedure was repeated for a predetermined number (8-10) of cycles.

EXPERIMENTAL RESULTS

Thermal Exposure

At a chamber temperature range of 26°-32°C (70% relative humidity), no significant colonic temperature rise was observed during a 15-min exposure period. At 35°C, however, rat #3 had a 0.3°C rise. (Threshold values among individual rats showed considerable variation.)

Figure 1 shows the colonic temperature (rat #3) rise time and down time for a 1°C cycle at a chamber temperature of 45°C. The rise time was relatively constant during five cycling periods but the down time varied considerably. During the first two cycles, down time was in the 25-26-min range; but during the next two cycles, it was considerably reduced. Thermal and RFR exposure of other rats showed similar patterns. This feature was peculiar to the unanesthetized rat: the reduction in down time did not occur in anesthetized rats.

Because the effect of environmental temperature on the cycling process was of considerable interest, rats were exposed at various chamber temperatures, with rise and down times measured. Figure 2 shows that at 40°C, the rise time (rat #1), was relatively high but declined as the chamber

temperature was raised. Down time was also initially high, then passed a minimum at the 44°C range and subsequently increased again. Since the average chamber temperature is constant, rise time is proportioned to the total thermal energy input into the rat. In general, the ratio RT/D (T = absolute chamber temperature) is an index of heat dissipation of the exposed rat (loss of calories per unit time). At chamber temperatures above 55°C, rat #1 became very restive and its temperature rise was very rapid (data not presented on Fig. 2). Initially (at 40°-44°) the heat-dissipation efficiency was relatively high, and then it declined to a relatively low level at 55°C; however, Figure 1 data indicates that repeated cycling at constant temperature improves heat-dissipation efficiency.

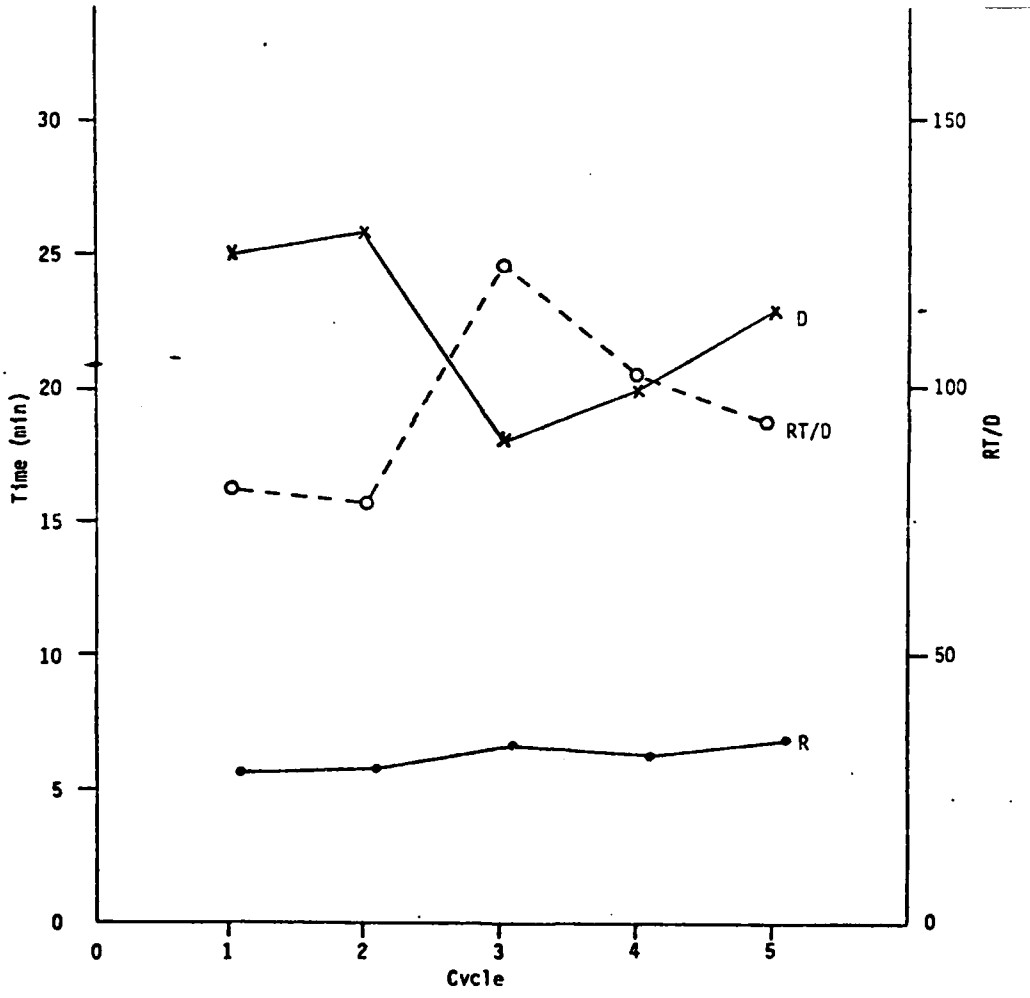

Figure 1. Temperature cycling of rat #3 in thermal chamber. Each cycle is comprised of a rise in colonic temperature from 37° to 38°C (R = rise time) and a return from 38° to 37°C (D = down time). RT/D is the heat-dissipation index in which T = absolute chamber temperature. Temperature rise was achieved by placing the rat in a thermal chamber at 45±2°C, 80% relative humidity.

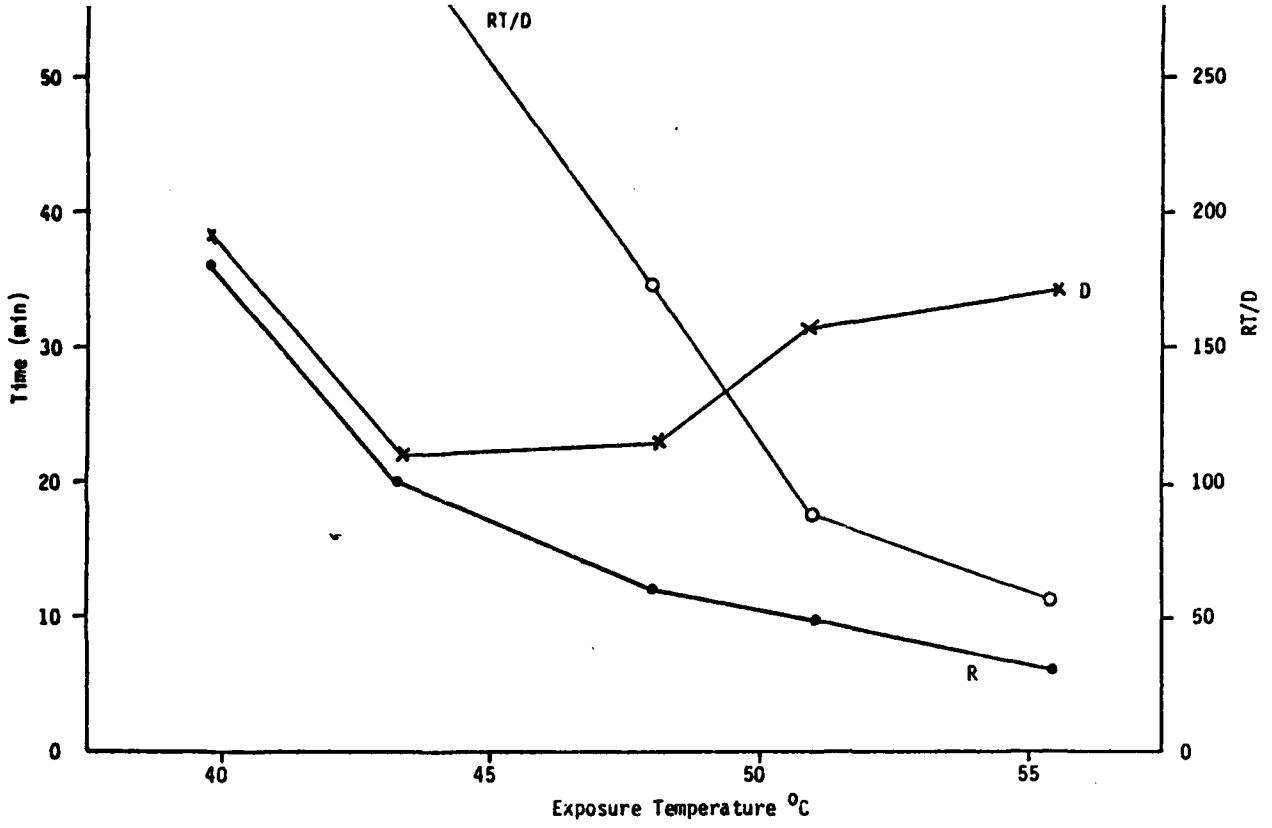

Figure 2. Rise and down time and heat-dissipation index as a function of thermal chamber temperature for rat #1. R = rise time; D = down time; RT/D = heat-dissipation index where T = absolute chamber temperature. Left ordinate: rise and down time in minutes; right ordinate: heat-dissipation index RT/D. Cycling temperature range was 37.5° to 38.5°C, colonic; chamber relative humidity, 80%.

RFR Exposure

At the beginning of our program, we had no information on rates of temperature rise and heat dissipation in the rat; therefore, we used substantial RFR power levels so the phenomena could be more easily observed. Figure 3 shows temperature cycling due to a pulsed field (1.0-ms pulse, 500 pps) at 100-mW/cm^2 power density level with a carrier frequency of 2.06 GHz. Cycles 9 and 10 had 1.3- and 1.4-min rise times, respectively, for a 2^o temperature rise; cycles 11 and 12 (2.0-min rise time for each cycle) represent a 3^{o}C temperature change from basal level. The temperature decline was initially rapid when the power was turned off, but the rate was considerably reduced when the temperature approached the basal level. Recordings made at higher speeds indicated a reduction in the rat's core temperature within 3-5 seconds after the field was turned off.

All rats, however, were not similar in terms of temperature rise and decline kinetics. Figure 4 shows rate processes that are almost linear. The rat was exposed to pulsed RFR (1.0-ms pulse, 500 pps). The average power density was 100 mW/cm^2 with a 2.06-GHz carrier frequency. Here the rise time for cycle 4 was 3.0 min for a 2^{o}C temperature rise. Subsequent data revealed that the curve form for temperature cycles depends on a variety of factors such as power density level, rat basal temperature, and effect of drugs; but the curve form also seems to depend on the inherent physiological status of the particular rat. At lower power densities, such as 50 mW/cm^2, the temperature rise and decline are gradual and almost linear. Cycling is slow and nonuniform at 30 mW/cm^2 and is absent at 10 mW/cm^2.

Effect of Prolonged Temperature Cycling

We originally proposed that to separate thermal from nonthermal effects, a long RFR exposure would be needed to produce some physiological changes due to nonthermal origin. We also considered that temperature regulatory mechanisms could be affected by fields and may exhibit changes in rise- and down-time patterns. Therefore, prolonged temperature cycling, where nonthermal injury or alteration would be cumulative, could have a negative impact on the thermoregulation process. This impact might be expressed in terms of reduced heat-dissipation efficiency, which would be exhibited by lengthened down time. Therefore, rats were exposed to a relatively high field density (100 mW/cm^2) and cycling was continued for a 6-7 hour period at 1^o, 2^o, and 3^{o}C levels.

Figure 5A shows the rise and down times of rat #5 during prolonged cycling induced by intermittent exposure to pulsed RFR (1.0-ms pulse, 500 pps). The average power density was 100 mW/cm^2 with a 2.06-GHz carrier frequency. Cycles 2-11 were carried out at a 2^{o}C increment, and cycles 12-18 at a 3^{o}C increment. Rise time gradually increased during cycles 2-7, then declined and stabilized (cycles 8-11). The increase in rise time indicates that the rat was resisting the temperature rise. In contrast, the down time decreased during the initial phase (cycles 2-4), but subsequently reached a steady fluctuating pattern. The decrease in down time indicates that the rat had become more effective in heat dissipation. Rise and down times at 3^{o}C cycling were respectively longer, as would be expected.

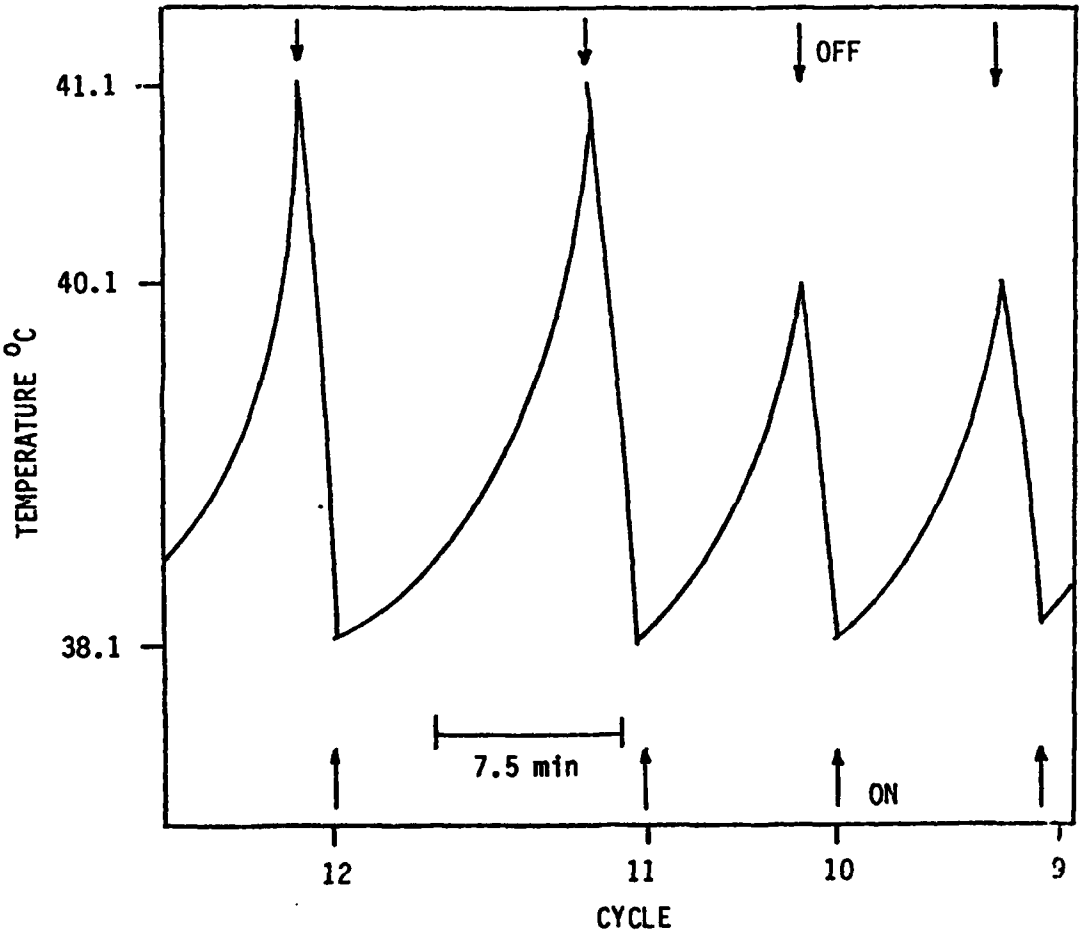

Figure 3. Temperature cycling of rat #2 exposed to pulsed RFR field (1-ms pulse, 500 pps). Carrier frequency is 2.06 GHz with average power 100 mW/cm^2. Temperature cycling at 2° and 3°C, from 38.1°C basal level. From the right, successive cycles are 9 and 10 at 2°C rise and 11 and 12 at 3°C rise. Animal was placed in the field in K orientation. Chamber temperature was 24°C; relative humidity, 77%. ON (↑) and OFF (↓) arrows indicate the power on and off times.

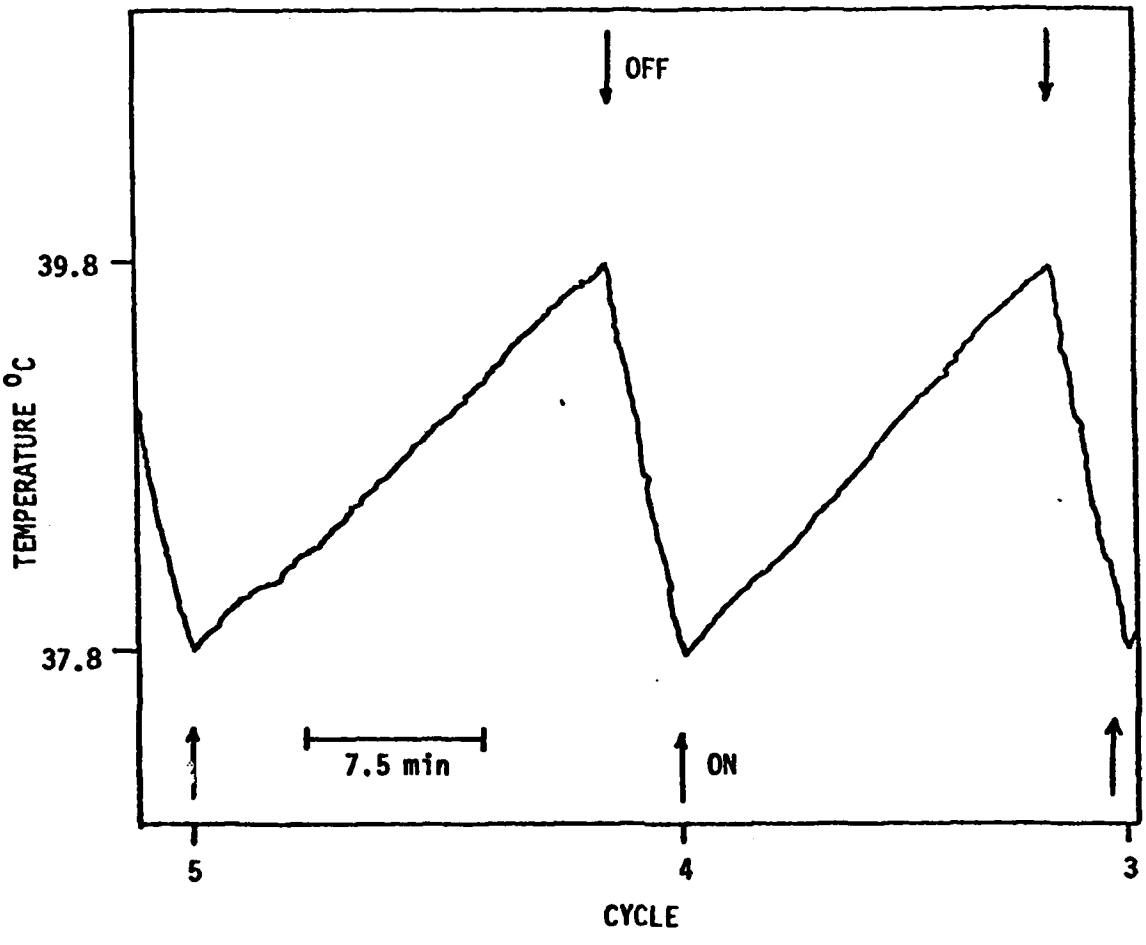

Figure 4. Temperature (2°C) cycling of rat #5 exposed to pulsed RFR field. Radiation conditions as described in legend of Figure 3. Basal temperature, 37.8°C.

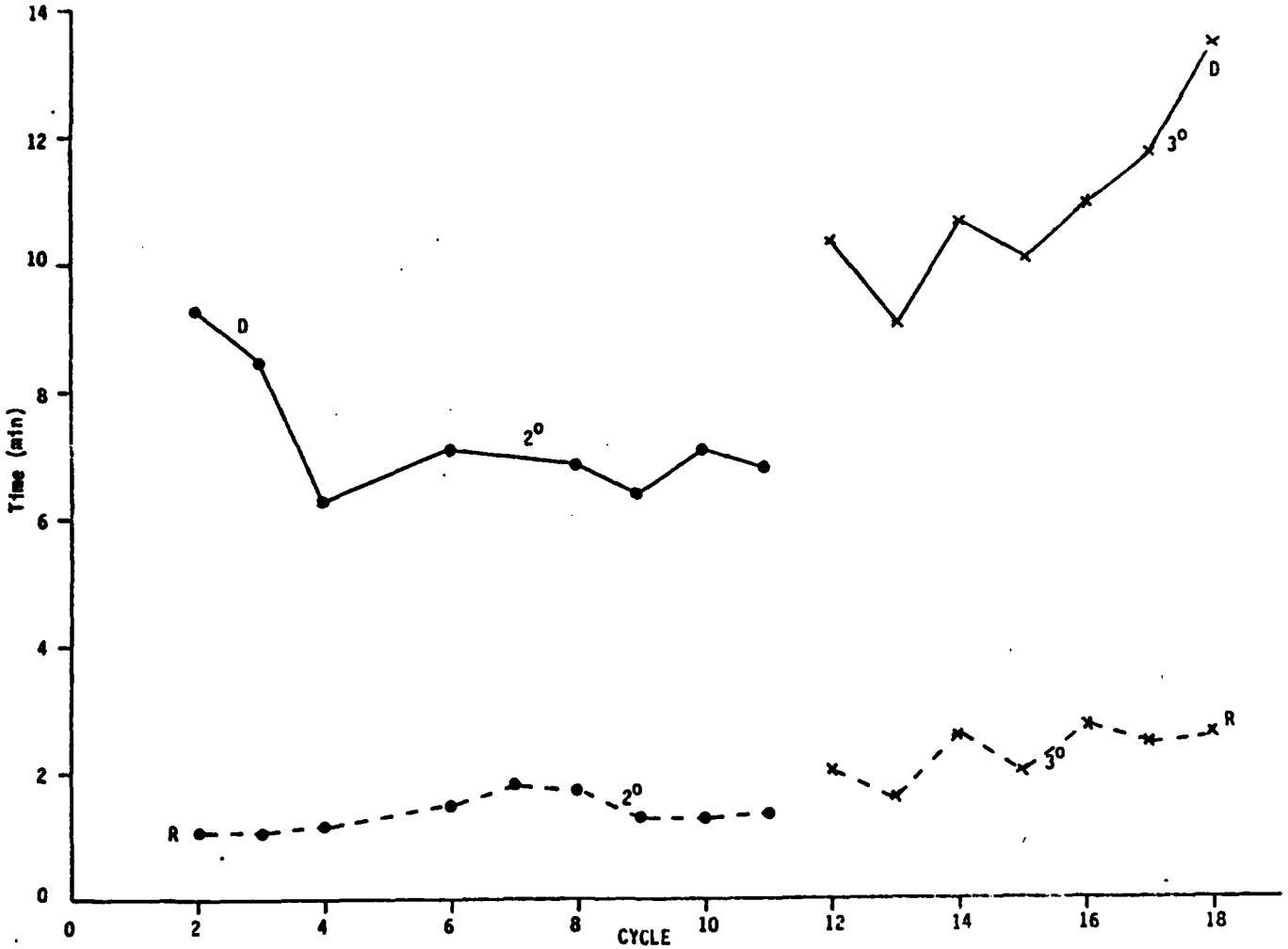

Figure 5A. Temperature rise (R) and down times (D) as a function of sequential cycling. Cycles 2-11, 2°C temperature rise; cycles 12-18, 3°C. Field frequency, 2.06 GHz; average power density, 100 mW/cm^2; pulse width, 1 ms; repetition rate, 500 pps. Animal in K orientation. Chamber temperature, 24°C; relative humidity, 77%.

However, the meaning, if any, of a considerably longer down time during cycles 16-18, is not clear.

We can evaluate the efficiency of thermal cycling on two premises:

1. The ratio R/D is an index of heat dissipation at a specific cycling temperature and increment (for example, a 1^{o}, 2^{o} increment, etc.). At a $37.0^{o}C$ cycling temperature and a $1^{o}C$ increment (i.e., cycle between $37.0^{o}C$ and $38.0^{o}C$), R represents the energy input necessary to increase the rat's temperature from $37.0^{o}C$ to $38.0^{o}C$ and D represents energy dissipation (when the input source is off) as the rat's temperature returns to $37.0^{o}C$.

2. The ratio R/C, where C = R + D, indicates the total heat-dissipation index. Energy is absorbed during the R phase and dissipated during the total cycle. Both indices have some merit, but which is the better index of efficiency is not clear yet.

The product, PR, where P is power density, is an entity that is proportional to the total energy absorbed during the cycle. The ratio PR/D is therefore an index of total heat dissipation during the cooling phase at a certain temperature increment. (This index is similar to RT/D which represented heat dissipation in the thermal chamber studies.) Figure 5B, showing PR/D as a function of cycles, indicates that in cycles 16-18, heat dissipation did not change during prolonged cycling. During the initial cycles the rat was less efficient, but thermoregulation improved until a typical fluctuating pattern was reached.

To reduce the thermal stress on the animal, the cycling temperature was reduced to $1^{o}C$, with cycling still performed for 6-7 hrs. Figure 6 shows the 1^{o} and 2^{o} cycling efficiency of rat #3. After 23 cycles the efficiency had increased substantially during the 1^{o} cycling; at the end of 2^{o} cycling, the efficiency had decreased from the initial level but was still greater than the initial efficiency level of 1^{o} cycling. The cycling period of this rat was one of the longest used in the experiments. Cumulative RFR exposure during the multiple cycling was 1.04 $W \cdot min/cm^{2}$.

Detection of Small Temperature Increments During RFR Exposure

To determine whether or not the rat is capable of performing temperature cycling at increments smaller than $1^{o}C$ is important not only in terms of RFR effects, but also when investigating the sensitivity of thermoregulatory mechanisms in general.

Conventional heat application techniques are not practical for inducing rapid and small incremental temperature changes in animals. Such changes are induced easily by RFR exposures since the field can be applied rapidly in small increments at varied power density levels. In our experiments temperature cycling was more effective when colonic temperature was raised a few degrees above basal temperature. When the rat was at basal temperature and was exposed to RFR to produce a temperature rise of $0.2^{o}C$, its temperature remained constant when the field was turned off. This also occurred when

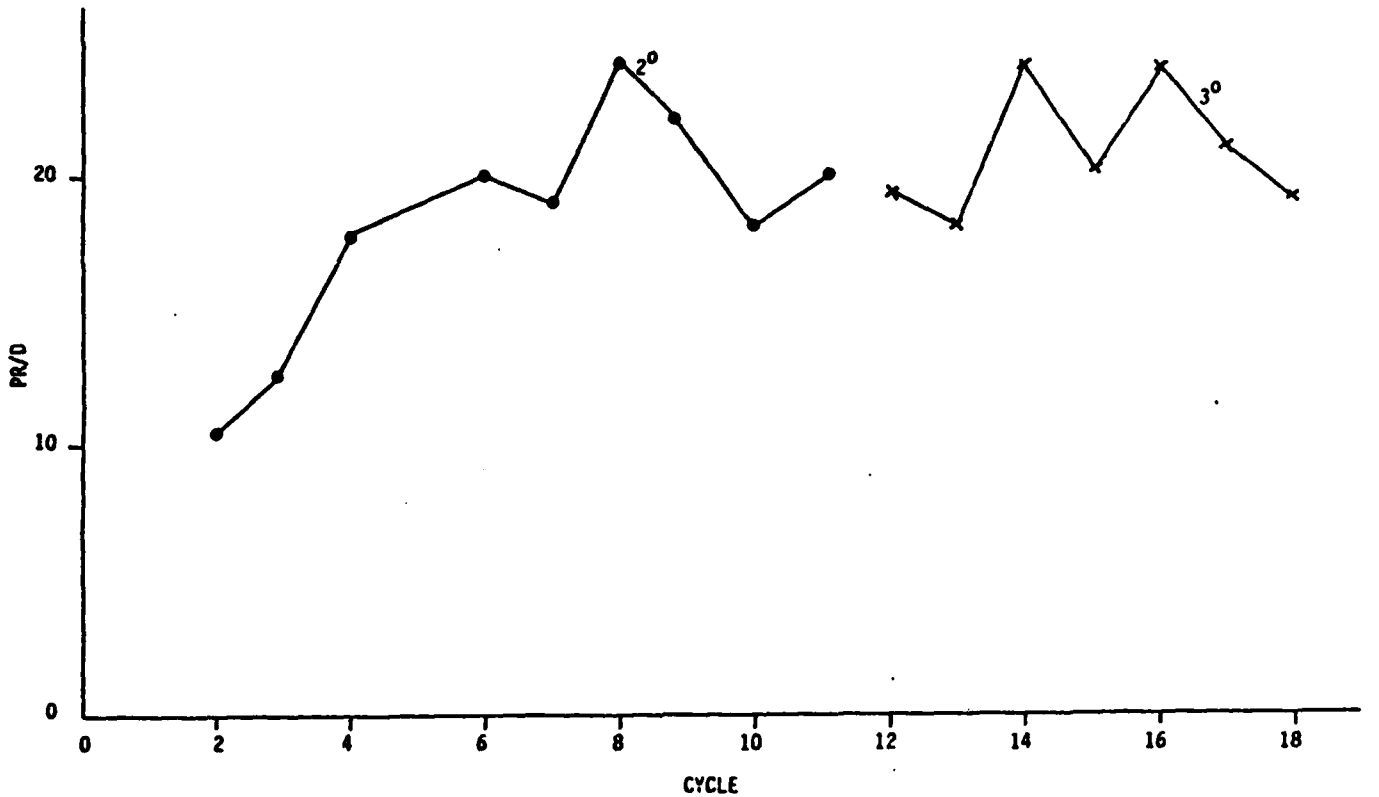

Figure 5B. Heat-dissipation index as a function of sequential cycling. Same experimental conditions as described in Figure 5A legend. RP/D is an index of heat dissipation where R is rise time, D is down time, and P is the power density.

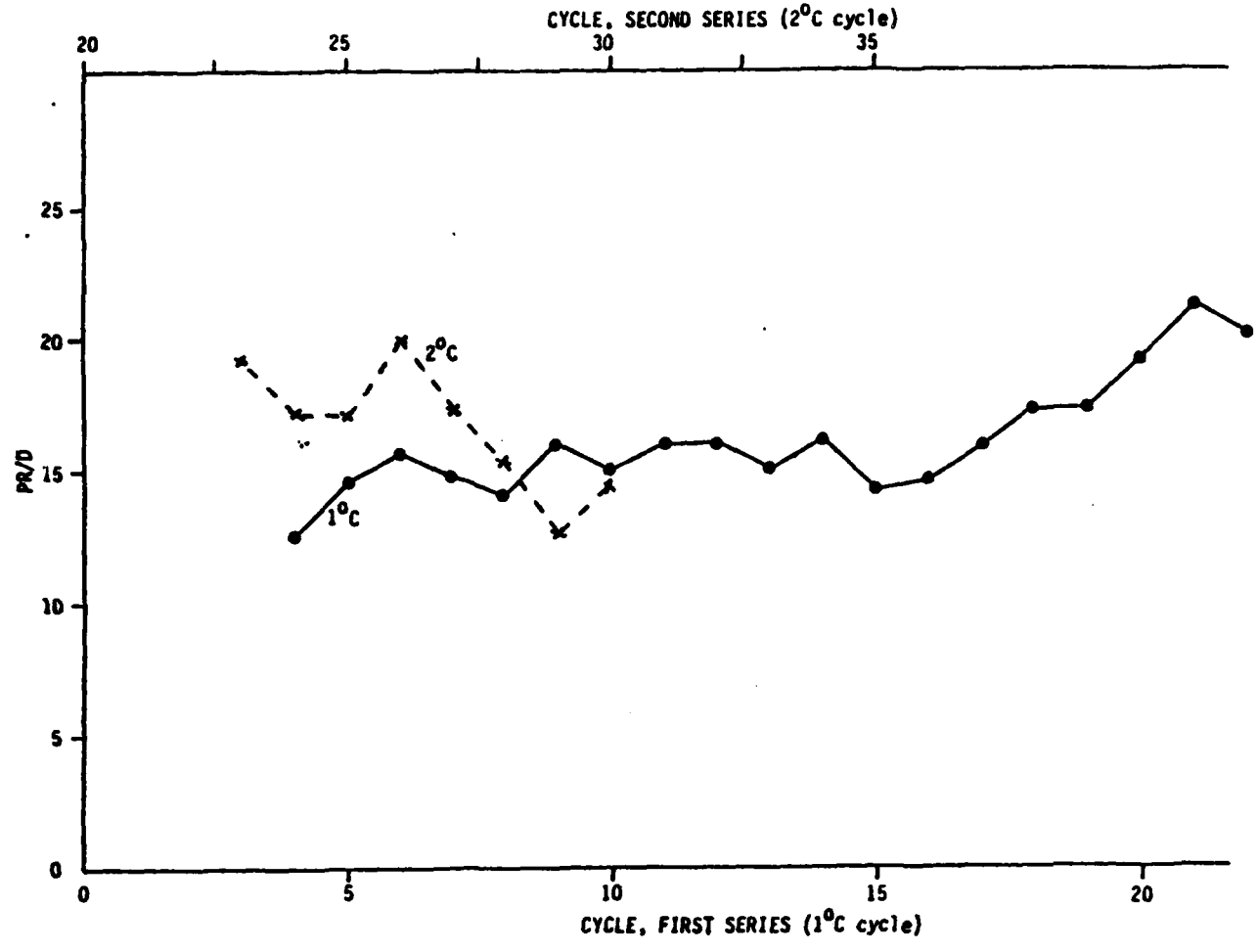

Figure 6. Heat-dissipation index in thermoregulation of rat #3 exposed to RFR during prolonged periods of temperature cycling.

First series of cycling lasted 23 periods at $1^{o}C$ change. (First 3 cycles were discarded due to animal agitation and movements.) In the second phase, cycling was continued at $2^{o}C$ increments to 30 cycles (upper cycling scale represents this period). Frequency, 2.06 GHz; pulse duration, 10 ms at 40 pps. Rat in K orientation facing the horn. Field power density, 100 mW/cm^2; total exposure, 1.04 $W \cdot min/cm^2$.

0.4° and 0.5°C temperature rises were produced. However, when the temperature rise was 1°C, the rat's temperature went down when the RFR exposure was discontinued. These experiments were carried out at a power density of 60 mW/cm^2 and with relatively long cycle times. The quantitative responses of different rats varied but the phenomenon was observed repeatedly. Because of this observation, we decided to set the rat's basal temperature to a higher level and see if its temperature would remain elevated after an RFR-induced 0.2°C rise. We found that with its basal temperature elevated to 40°C, the rat did not sustain a 0.2°C increase when the RFR field was turned off. The temperature fell until the field was reapplied, resulting in cycling. These observations were repeated with 0.4°, 0.6°, and 1.1°C reductions from the 40°level (Fig. 7). At each test level the rat was challenged with a 0.2°C RFR-induced temperature increase. When the field was turned off at any of these levels, the rat's temperature fell and cycling occurred. Similar results were obtained at lower temperatures. Figure 7 shows rat #4's cycling response in a range between 37° and 40°C. These experiments showed that the rat was able to regulate its temperature after a 0.2°C rise within the 37°-40°C range.

Effects of Chronic Exposure to RFR

Some limited observations made during these pilot studies are considered to be of phenomenological significance, but further quantitative studies are needed to substantiate the issues. Each rat was used repeatedly in a variety of temperature-cycling experiments during initial experimentation. Several rats therefore accumulated a very high total exposure time to RFR during the months of experimentation.

Figure 8 shows down time as a function of days. In a variety of experiments, rat #3 was exposed to CW and pulsed fields at a power density of 100 mW/cm^2, 2.06-GHz carrier frequency, during 1° and 2°C temperature cycling. Pulse duration was 1.0 ms (500 pps). The down time for 1°C temperature cycles was considerably lengthened during the experimental period. The down time for 2° temperature cycles also gradually increased, finally reaching a stable level. The data indicate that the effect on the rat's ability to dissipate heat may have been cumulative. After 40 days rest, downtime was considerably lowered during 1°C cycling. This cumulative effect has been observed in other rats with a more limited number of cycling days. When our experiments were organized and performed, we had no idea that such a phenomenon was going to occur; therefore, data collection took place from existing records when the series was finished. Since the cumulative effect is highly significant in terms of chronic RFR exposure effects on thermal regulation, it also may have significance in the alteration of other physiological processes. Further systematic studies are desirable.

After completion of the experimental series, the weights of 3 rats (#3, #4, and #5) were plotted as a function of days after the start of the experimental period. Figures 9A, B, and C show that these weight-change patterns were similar to those of normal rats used by other investigators (13). RFR had no consistent effects on weight change.

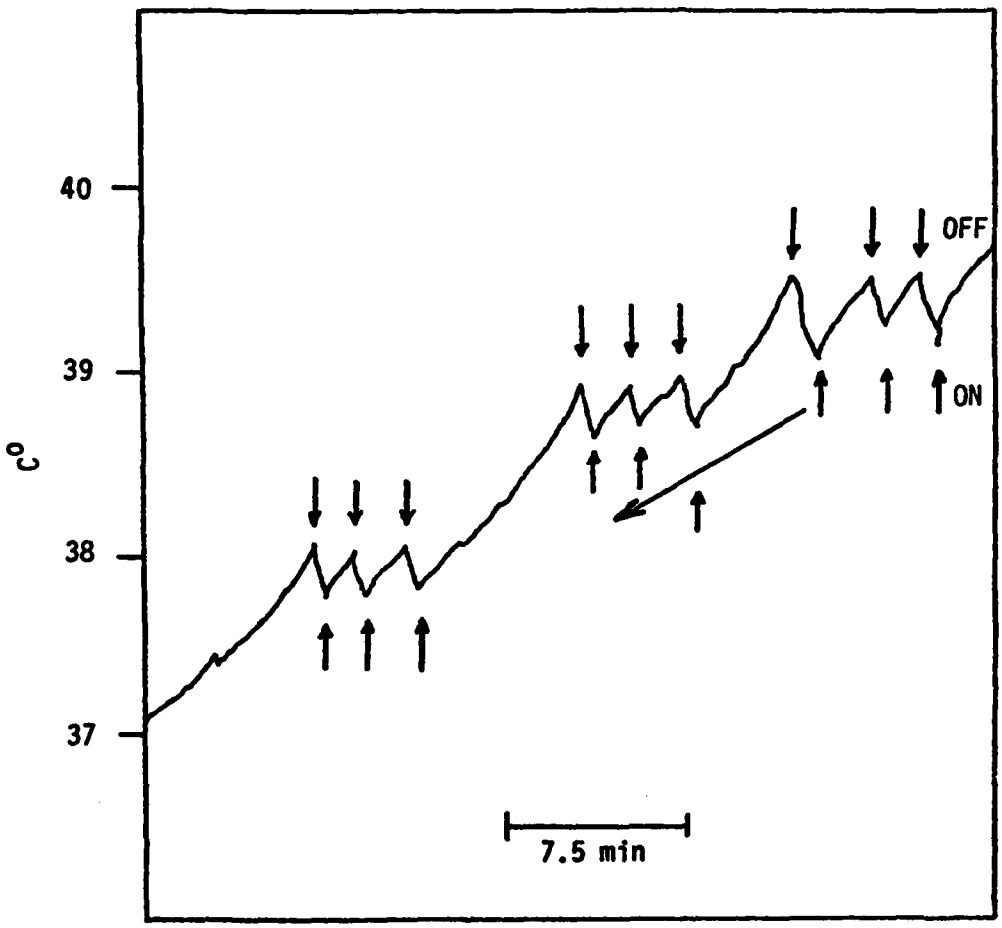

Figure 7. Effect of starting temperature on cycling. Rat #4 responds to 0.2°C RFR-induced temperature increases by reducing temperature toward baseline when field is off at 0.4°, 0.6°, and 1.1°C increment reductions from 40°C. Cycling is shown at each level. RFR CW (2.06 GHz) exposure at 60 mW/cm² power density. Field ON (↑) and OFF (↓) as indicated at arrows. Large, diagonal arrow shows direction of experiment progression.

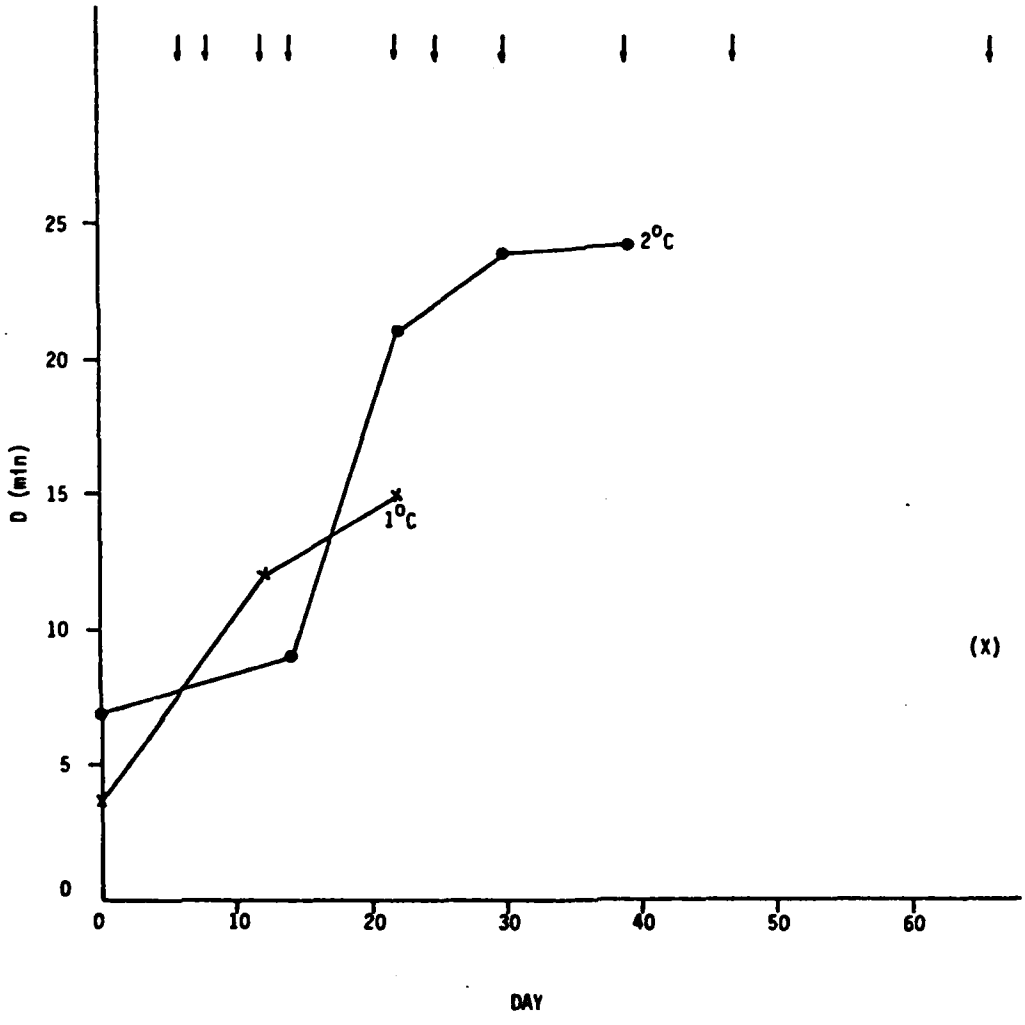

Figure 8. Down time during chronic exposures of rat #3. Graph represents exposure densities of 100 mW/cm^2 at 1^o and 2^{o}C temperature cycling. The rat was exposed to both pulsed and CW radiation at 2.06-GHz carrier frequency. Pulse duration was 1.0 ms (500 pps). During a 66-day time span, the rat received additional RFR exposure in other procedures. Arrows indicate days when the rat was irradiated. Total exposure was 42.9 W•min/cm^2. (X) indicates 1^{o}C cycling of rat after rest period.

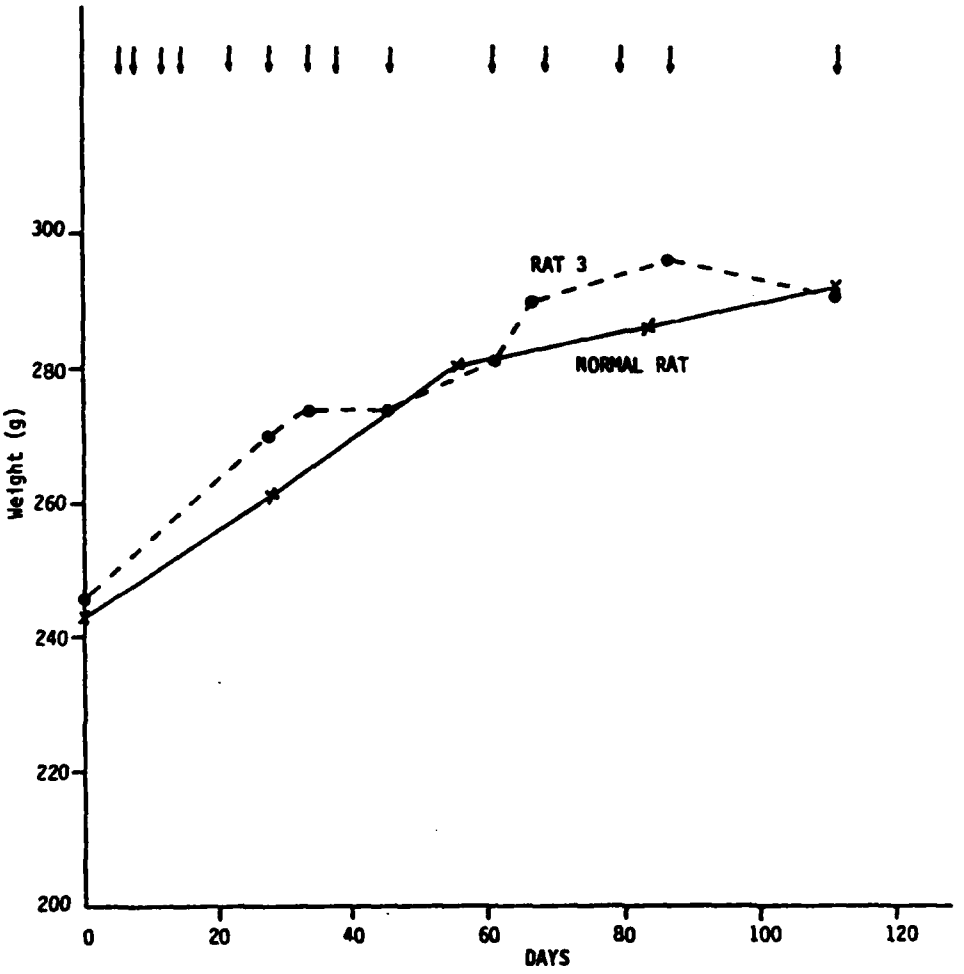

Figure 9A. Weight fluctuation of rat #3 during chronic RFR exposure compared with that of a normal rat with similar initial weight. Arrows indicate days of exposure. Total accumulated RFR exposure was 42.9 W-min/cm^2. Normal-rat data from growth table for laboratory animals (7).

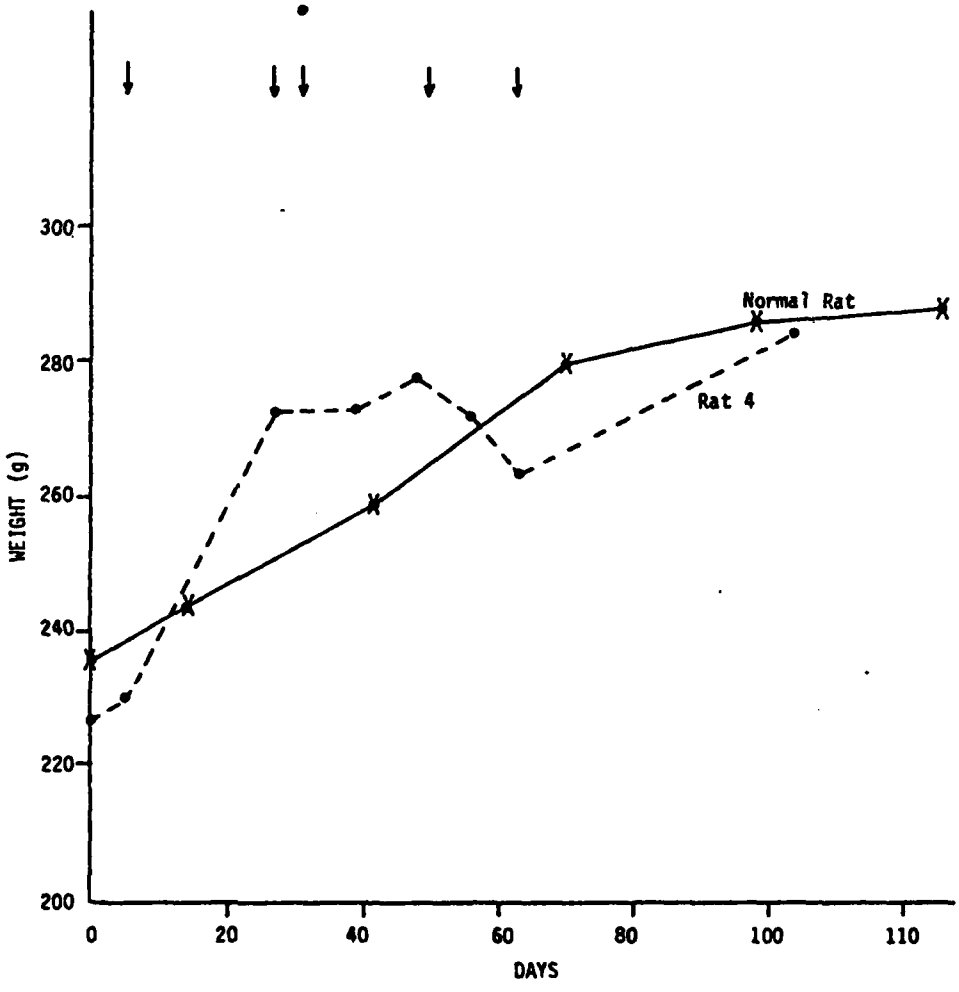

Figure 9B. Weight fluctuation of rat #4 during chronic RFR exposure compared with that of a normal rat with similar initial weight. Arrows indicate days of exposure. Total accumulated RFR exposure was 14.8 W·min/cm^2. Normal-rat data from growth table for laboratory animals (7).

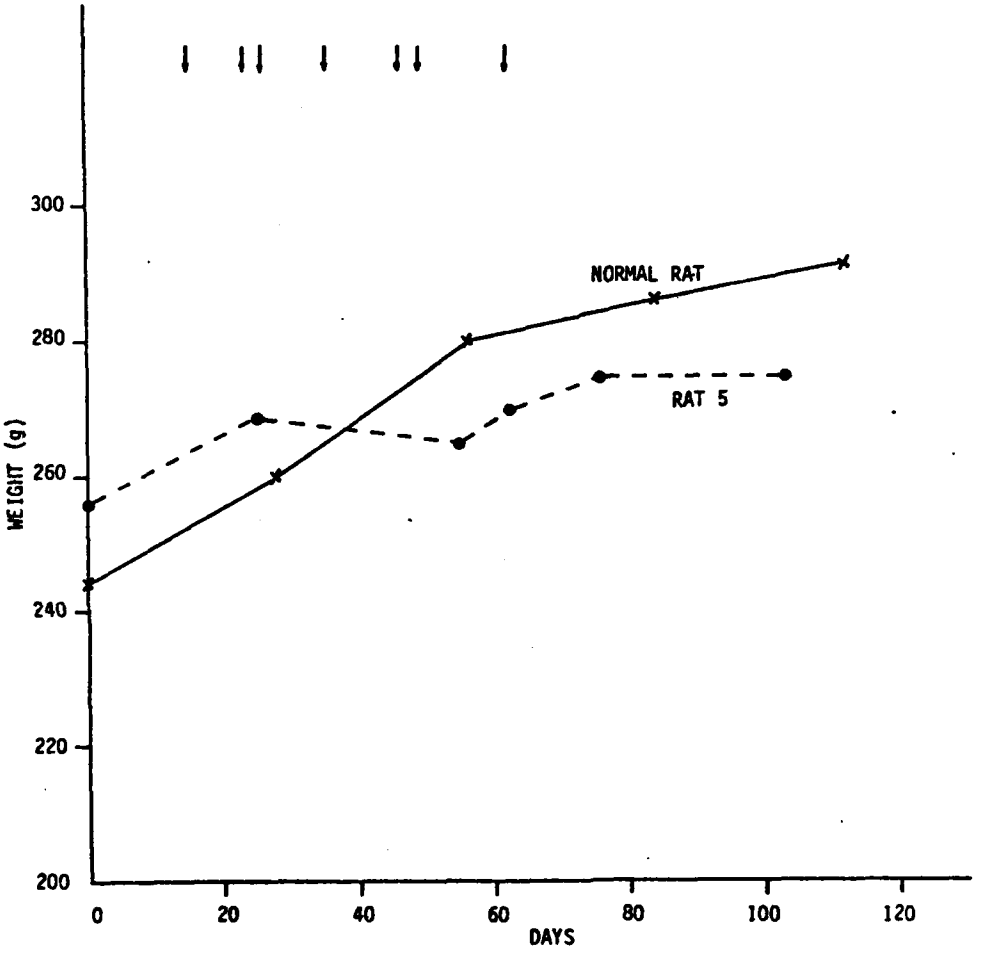

Figure 9C. Weight fluctuations of rat #5 during chronic RFR exposure compared with that of a normal rat with similar initial weight. Arrows indicate days of exposure. Total accumulated RFR exposure was 28.1 W·min/cm^2. Normal-rat data from growth table for laboratory animals (7).

Temperature Cycling at Various RFR Power Density Levels

investigate whether or not thermoregulatory processes are affected by ing thermal load, experiments were performed at various power levels 50 and 200 mW/cm^2. Below 50 mW/cm^2, rats cycle slowly, so it was im- e to perform an adequate number of cycles per day. The upper limit of ensity was determined by the fact that thermal rise time became quite nd difficult to measure accurately. The rise time in the 50-200-mW/cm^2 ange decreased from 7.0 to 0.8 min as the power level was increased. re 10, curve 1 indicates the heat-dissipation efficiency index (PR/D: P r density; R, rise time; and D, down time) at 2.06-GHz CW and varied ensity levels. When the rat was alert, the heat-dissipation index was ely high at lower power densities (50-75 mW/cm^2). At higher power den- (75-200 mW/cm^2), the index was lower but stabilized at a fairly con- evel. Curve 2 reveals the heat-dissipation index for the same rat, entical RFR exposure conditions, under ketamine anesthesia. Here, no cant power density effect was evident. Similar effects have been ob- with several other rats.

other preliminary observation is that the rat's heat-dissipation effi- index may improve with repeated cycling trials. After exposures at 50, 0, and 200 mW/cm^2 power densities, another trial at 100 mW/cm^2 was per- This PR/D (Fig. 10, circled x), showed a 62% increase when compared e earlier trial at 100 mW/cm^2. Similar results were seen with other Further studies should determine if the increase in PR/D is due to re- trials, to a preservation of the thermoregulatory response necessary at effects of RFR exposure at higher power densities, or to other . These studies should include altering the sequence of low and high ensity exposures.

Comparison Between Pulsed and Continuous-Wave Microwave Field Effects on Temperature Cycling

nce CW and pulsed RFR may produce different energy gradients in tissue ructures, the mode of RFR application may alter the characteristics of dissipation. Whether this is of practical significance at the power used in the current experiments has yet to be demonstrated. Diffi- arise when trying to resolve such issues in experiments where only a umber of animals are available. However, a limited number of experi- ere made where each animal served as its own control. Three cycles ed RFR were followed by three cycles of CW exposure. This sequence formed twice during a 6-hr period. Rat #2 was exposed to 2.06-GHz CW sed field (500 pps, 10-ms pulse width). In both cases the average ensity was 100 mW/cm^2. The mean ($\pm$SE) heat-dissipation indices (R/C: se time; C, total cycle time, i.e., R + D) for CW and pulsed exposure 130 $\pm$ 0.032 and 0.29 $\pm$ 0.026, respectively. Experiments with rat #3 similar results. In another series of experiments, the pulse width ied from 1 to 10 ms while the average power level remained constant; results were obtained. No drastic difference was observed between CW sed-field effects on the efficiency of heat dissipation during temper- ycling. To obtain more definitive comparative data, experiments be performed with a larger pulse peak power and measurement of addi- experimental parameters. Such experiments are in progress.

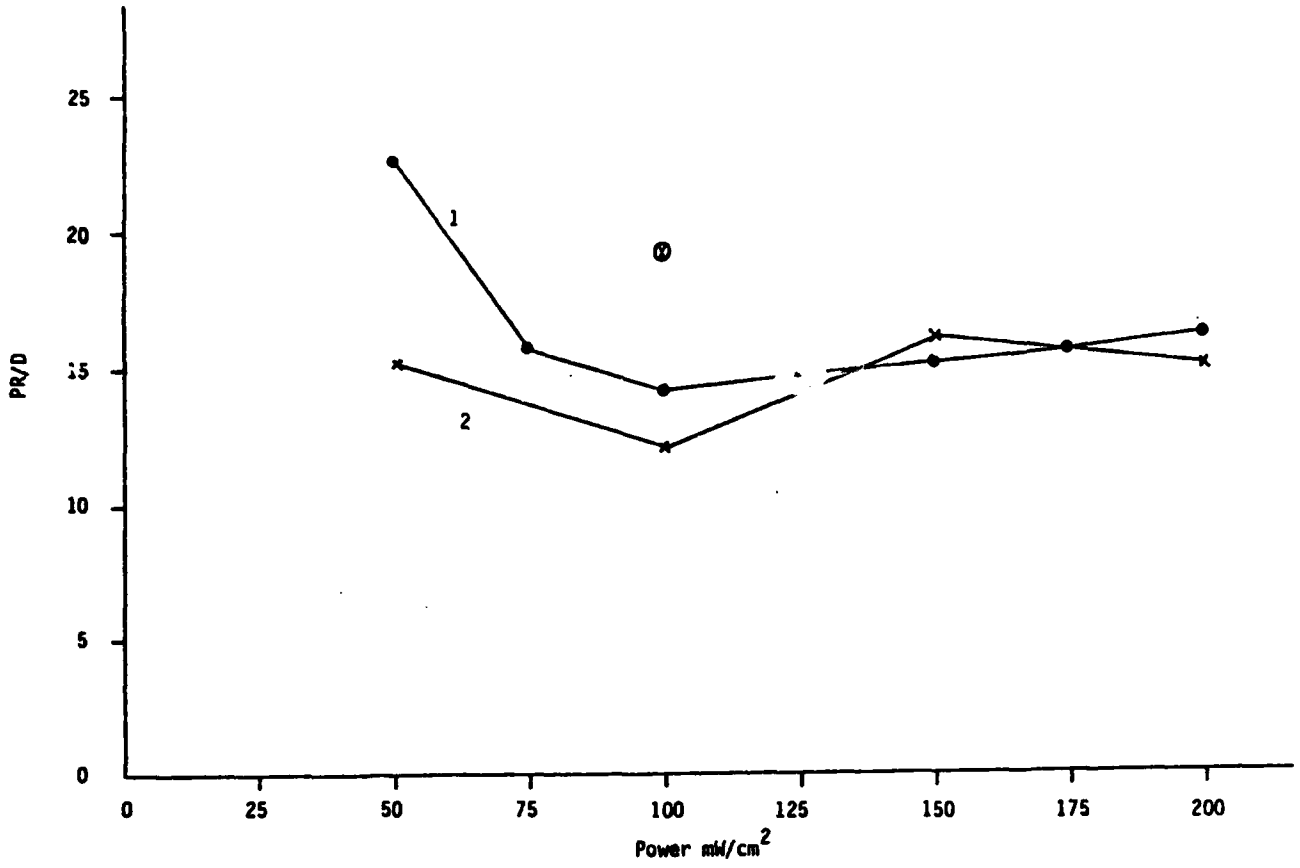

Figure 10. Effect of ketamine anesthesia on the heat-dissipation index (PR/D). Rat #3 exposed to 2.06 GHz, CW field in E orientation at varied power density levels. Curve 1 shows PR/D (1^0 cycling) as a function of power density for an alert rat. Curve 2 shows the PR/D for the same rat under ketamine anesthesia (100 mg/kg, IP); ⊗ indicates PR/D at second 100-mW/cm^2 trial in ketamine-treated rat. This experiment was performed 1 week after the alert study.

Effect of Field Orientation on Temperature Cycling

We performed a series of preliminary experiments to determine if the orientation of the animal within the field affected the animals' thermal response when the field was applied. Because of the many possible combinations of power and pulse characteristics, anesthetics, and orientations, we arbitrarily selected conditions similar to those used in other parts of our overall study of RFR bioeffects. Experiments were performed with two rats so that the effect of K and H orientation could be examined in more detail. This was motivated by the fact that thermal energy distribution in the rat during field exposure depends not only on general orientation, but also on which part of the body and organs receive the main thermal insult. Figure 11 reveals the heat-dissipation index at various body orientations for rats with 1°C temperature cycling induced by exposure to CW RFR at 2.06 GHz with a 50-mW/cm^2 power density.

In addition to the above studies, 18 preliminary observations were made with unanesthetized rats in the K (N = 11), E (N = 2), and H (N = 5) orientations (N = number of experiments) and exposed to 2.06-GHz RFR with varied power and pulse characteristics (CW: N = 10, range 50-200 mW/cm^2; pulsed RFR: N = 7 at 100 mW/cm^2, 500 pps, 1-ms pulse width, and N = 1 at 50 mW/cm^2, 20 pps, 10-ms pulse width, and N = 1 at 100 mW/cm^2, 40 pps, 10-ms pulse width). Also, 21 preliminary observations were made with rats anesthetized with ketamine (N = 11), ketamine-valium (N = 5), and acepromazine (N = 5). These animals were exposed to 2.06-GHz CW RFR (range: 50-200 mW/cm^2, N = 6) and to 2.06-GHz pulsed RFR (100 mW/cm^2, 500 pps, 1-ms pulse width, N = 15). These preliminary studies indicate that temperature-cycling characteristics may vary with the orientation of the animal within the field. Subjective observations of behavior indicate that unanesthetized rats in the K orientation with head toward the horn were the least active of the alert rats studied.

DISCUSSION

Comparison of direct thermal- and RFR-induced cycling reveals that, as expected, the temperature rise time during exposure depends on energy input. Rise time was shortened when energy density (RFR, mW/cm^2) or environmental temperature was raised. However, the heat-dissipation indices during increased environmental temperature and RFR were not similar. At the 40°-45°C range of environmental temperature, the heat-dissipation index (RT/D; Fig.2) seemed to remain constant; but at higher temperatures it declined rapidly. The rate of decline in the RT/D was reduced at higher temperatures, but the significance of this is not clear since this change occurred at a level not within the physiologically tolerable temperature range. During RFR the heat-dissipation index (PR/D; Fig. 10) also was reduced when the power density increased, but it subsequently stabilized at higher power levels.

Differences of physiological regulatory responses to RFR and environmental temperature were not unexpected since energy entry and distribution differ.

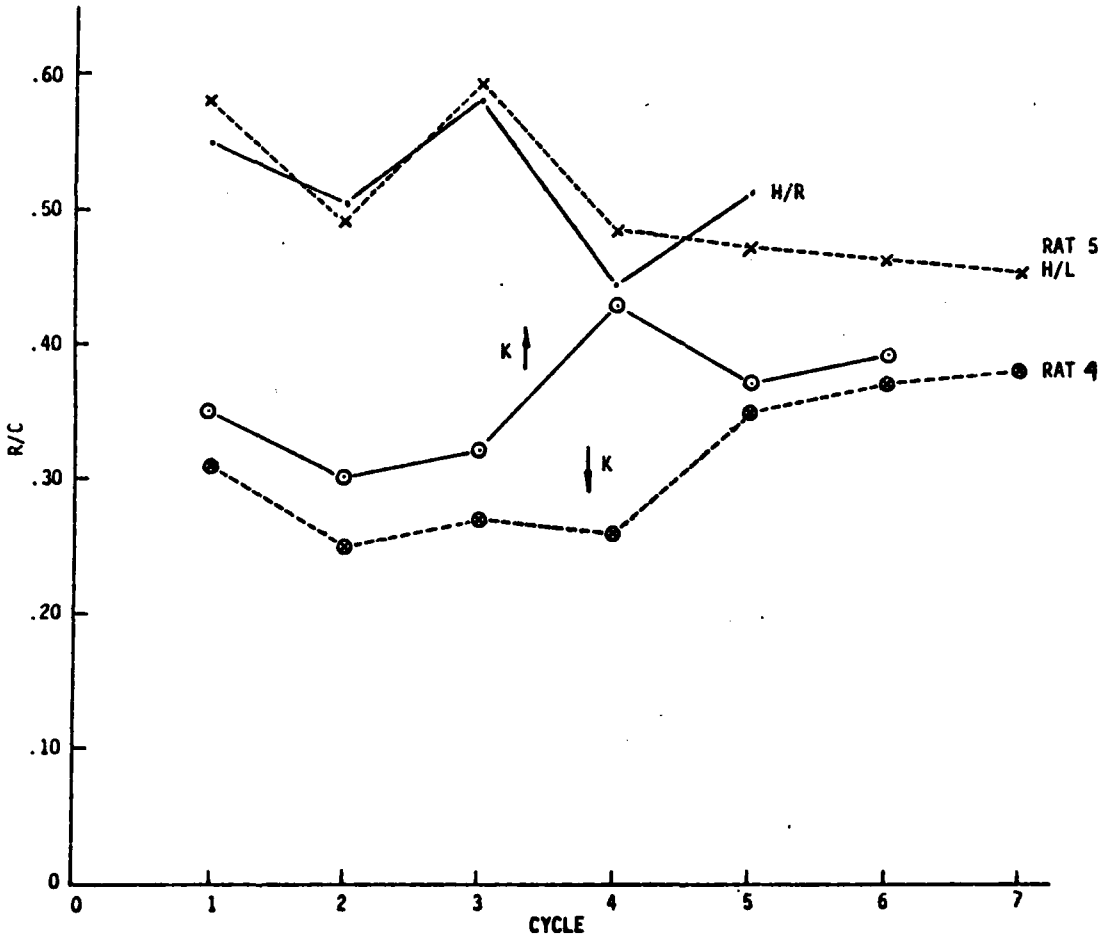

Figure 11. Effect of the rat's orientation in the RFR field. (R is rise time; C, time for the total cycle.)

Rat #4, exposed in K orientation: K↑, head toward horn; K↓, head away from horn.
Rat #5, exposed in H orientation: H/R, right side toward the horn; H/L, left side toward the horn.

Both rats were exposed to CW and RFR at 2.06 GHz, 50-mW/cm^2 power density. Temperature cycling was at 1^{o}C.

Also, sensory perception of these two energy forms may differ. The rat is capable of responding in regulatory terms to relatively small RFR-induced temperature increments (0.2°C, Fig. 7), indicating a high sensitivity of internal temperature sensors. Furthermore, the orientation of the animal in the field seems to be an important factor in temperature sensing and thermal regulation (Fig. 11) because internal energy distributions may differ; consequently, different local topographical temperatures could exist. It has been pointed out that "A uniform RF field incident upon an object does not generally produce a uniform RF internal field."(13) In the RFR fields studied, the K orientation may provide a more uniform energy distribution within the rat's body. Therefore, the rat may be less likely to attempt to change position to relieve possible discomfort resulting from uneven heating.

These findings may be helpful in designing future experiments using alert rats, because sporadic movements during exposure interfere with certain physiological recording procedures (e.g., electrocardiography). The findings point out a need for studies of specific absorption rates in rats at the RFR characteristics used in these studies. This group of experiments also shows that use of proper anesthesia (one that does not interfere with temperature regulation and cycling) allows selection of animal orientation.

An important issue in RFR exposure is comparing the bioeffects of CW fields with those of pulsed fields. Our experiments, with a limited number of rats and using the 1-10-ms pulse range (500 pps, 2.06 GHz, average power density 100 mW/cm^2), revealed no significant differences between the effects of these two types of fields on temperature cycling in rats; also, that thermoregulatory mechanisms are not drastically affected. However, these studies must be expanded to include different pulse characteristics as well as to study the effects on different physiological parameters. Studies are in progress for determining the effects of CW and pulsed RFR on arterial blood pressure, respiration, and cardiac function (measured by an electrocardiogram).

The main purpose of this series of pilot experiments was to determine whether or not temperature monitoring in conjunction with RFR exposure would permit exposing rats to a high dose of radiation energy without exceeding predetermined temperature levels. Our conclusion is that the methodology of temperature cycling is an effective means of introducing large amounts of radiation energy into the system while maintaining the animal within a physiologically acceptable temperature range. These cycling experiments were performed in the span of 4 months. Although a few changes were observed in the temperature response to RFR, the rats remained viable. As rats were rested for a period of time, their cycling time increased--returning to near initial levels. These experiments indicate a cumulative effect that could be caused by stress and fatigue or some regulatory impairment, but further studies are required to elucidate this phenomenon.

Advancing conclusions on the comparative bioeffects of CW and pulsed RFR from this preliminary study would be premature because of the limited spectrum of field and pulse characteristics used and the number of observations made.

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